

# Pancreatic function and histoarchitecture in Wistar rats following chronic exposure to Bushfire<sup>®</sup>: the mitigating role of zinc

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## Abstract

**Objectives:** To assess the toxicopathologic effects of chronic exposure to the glyphosate-based herbicide Bushfire<sup>®</sup> on the pancreas of Wistar rats and the protective role of zinc.

**Methods:** We exposed the rats to daily doses of 14.4 to 750 mg/kg body weight of the glyphosate-based herbicide Bushfire<sup>®</sup> and to 50 or 100 mg/kg zinc, and measured blood glucose levels and serum insulin levels. Tissue samples were evaluated for histopathological alterations.

**Results:** Levels of both blood glucose and serum insulin increased in glyphosate-exposed rats, and moderate to severe degenerative changes were observed in both glandular pancreatic acinar cells and islets of Langerhans in all rats exposed to glyphosate. These effects were prevented by pretreatment with zinc.

**Conclusion:** Chronic exposure to glyphosate can alter pancreatic function and histoarchitecture, but zinc supplementation can mitigate these toxicopathologic effects.

## Keywords

Glyphosate, zinc, mitigation, chronic, toxicopathology, pancreas, glucose, insulin

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## Introduction

Glyphosate (N-Phosphonomethyl-glycine) is a non-selective herbicide used worldwide to control weeds.<sup>1</sup> Glyphosate-based formulations for commercial uses are primarily made up of an aqueous mixture of glyphosate in the form of a salt, a surfactant, and various minor constituents.<sup>1</sup> Globally, glyphosate is the most widely used herbicide, and over 130 countries permit its extensive use; the US is the largest consumer, accounting for approximately 20% of the market.<sup>2</sup> Over the past few years, concerns have been raised that environmental exposure to glyphosate-based herbicides may cause endocrine disruption and organ damage at doses below regulatory limits.<sup>3–5</sup> Poisoning of domestic animals by pesticides and other agricultural chemicals is attributable to human error such as inaccuracies in calculating concentrations for spraying and dipping procedures, resulting in exposure to concentrations several times higher than recommended.<sup>6</sup>

The phytotoxicity of glyphosate is mediated by its action on various enzyme systems; the pesticide inhibits amino acid metabolism in what is known as the shikimic acid pathway.<sup>7,8</sup> Its toxic mechanism of action in animals is not clear, although laboratory experiments have suggested that the toxicity is due primarily to the presence of surfactants in the formulation, and oxidative stress is the indicated molecular mechanism of glyphosate toxicity.<sup>1,9</sup> Recent research has elucidated the toxicological effects of glyphosate-based herbicides in humans and animals.<sup>9–12</sup> Altered glucose homeostasis and oxidative impairment in the pancreas of rats exposed to the organophosphate insecticide dimethoate have been reported.<sup>13</sup> We previously found that zinc supplementation arrested glyphosate-mediated cellular degeneration in rat pancreas without altering the histoarchitecture of the organ.<sup>14</sup> There is still, however,

insufficient information on the effects of chronic glyphosate exposure on pancreas histology and function, or on the ameliorative effect of zinc.

Zinc is an essential trace element for a number of animal species. Under stress conditions, the liver synthesises large quantities of metallothionein, which then binds to zinc and can reduce its levels in the body, leading to a deficiency. Metallothionein is also synthesised by the non-glandular pancreatic acinar cells.<sup>15,16</sup> Zinc has been shown to slow or delay the oxidative process<sup>14</sup> by two mechanisms of action. The first mechanism involves the protection of sulphhydryl groups from oxidation via inhibition of intramolecular disulphide formation.<sup>13</sup> The second mechanism involves the prevention of free radical formation by transition metals.<sup>17–19</sup> Oxidative stress has also been implicated in the molecular mechanisms of glyphosate toxicity.<sup>14</sup> The objectives of this study were to investigate the effects of glyphosate on pancreas histology and function and to evaluate the mitigating role of zinc on alterations induced by chronic glyphosate exposure in rats.

## Materials and methods

### Animals

Approval of the study was obtained from the Ethics Committee on Animal Use and Care of Ahmadu Bello University (Zaria, Kaduna State, Nigeria). Eighty adult male Wistar rats weighing 140 to 150 g were purchased from the National Institute for Trypanosomosis and Onchocerciasis Research (Vom Office, Jos Plateau State, Nigeria). The animals were housed in the animal room of the Department of Veterinary Pathology, Ahmadu Bello University-Zaria for two weeks for acclimatisation prior to the experiment. The rats were fed standard rat chow and water was provided *ad libitum*.

## Chemicals

A glyphosate-based herbicide (Bushfire<sup>®</sup>) containing 360 g glyphosate/L in the form of 441 g/L potassium salt, distilled water, and zinc chloride (BDH Chemicals Ltd.; Poole, UK), haematoxylin and eosin stain, and aldehyde fuchsin stain were obtained from a reputable chemical store in Zaria.

## Experimental design

### Chronic toxicity study

The rats were randomised into eight groups of 10. Group I (DW) served as the control and received 2 mL/kg of distilled water daily. Group II (Z) received 50 mg/kg body weight zinc.<sup>20</sup> Group III (G1) received 14.4 mg/kg glyphosate (2% concentration in 2 mL of distilled water, the standard concentration used for agricultural spraying). Group IV (G2) received 375 mg/kg of the glyphosate-based herbicide Bushfire<sup>®</sup> (10% of the half-maximal lethal dose [LD<sub>50</sub>]).<sup>21</sup> Group V (G3) received 750 mg/kg Bushfire<sup>®</sup> (20% of the LD<sub>50</sub>).<sup>21</sup> Group VI (ZG1) was pretreated with zinc (50 mg/kg) and then administered Bushfire<sup>®</sup> (14.4 mg/kg) 1 hour later. Group VII (ZG2) was pretreated with zinc (50 mg/kg) and then administered Bushfire<sup>®</sup> (375 mg/kg) 1 hour later. Group VIII (ZG3) was pretreated with zinc (100 mg/kg) and then administered Bushfire<sup>®</sup> (750 mg/kg) 1 hour later.

The dose regimens were administered by gavage once daily for 36 weeks.<sup>22</sup> Rats were weighed weekly using a digital electronic balance (Hangzhou Gongheng, Hangzhou, China) to monitor weight changes and ensure appropriate dosing. No rats died during the experimental period.

### Determination of fasting blood glucose and insulin levels

Fasting blood glucose level was determined at the end of the study with a blood glucose

metre (Accu-Check<sup>®</sup>) using blood from the tail vein after fasting the rats overnight. Insulin was measured in serum using an ultrasensitive insulin ELISA kit (Monobind Inc., Lake Forest, CA, USA).

### Histopathological examination

Tissue samples from the pancreas were collected and fixed in 10% neutral buffered formalin. The samples were dehydrated in graded concentrations of alcohol (70%, 80%, 95%, and 100%), cleared using xylene, impregnated in paraffin wax, incubated in a vacuum oven at 60°C, embedded in plastic embedding rings, sectioned into 5- $\mu$ m slices using a microtome, deparaffinised with xylene, rehydrated in graded concentrations of alcohol (100%, 95%, 80%, and 70%), stained with haematoxylin and eosin,<sup>23</sup> and viewed under a light microscope. The histochemical features of the pancreas samples were also studied using aldehyde fuchsin staining.<sup>24</sup>

### Data analysis

Data are expressed as the mean  $\pm$  SEM and analysed by one-way ANOVA followed by Tukey's post-hoc test with GraphPad Prism version 4.0 for Windows (La Jolla, CA, USA).  $p < 0.05$  was considered statistically significant. Where there was no significant difference, the mean difference between groups, expressed as a percentage, is reported if the value was  $\geq 10\%$ .

## Results

### Effects of treatments on blood glucose levels

There was no significant difference ( $p > 0.05$ ) in blood glucose levels between the treatment groups. An increase in glucose levels was observed in the ZG3 and G3 groups compared with the levels in the

DW group (10% and 25%, respectively; Figure 1).

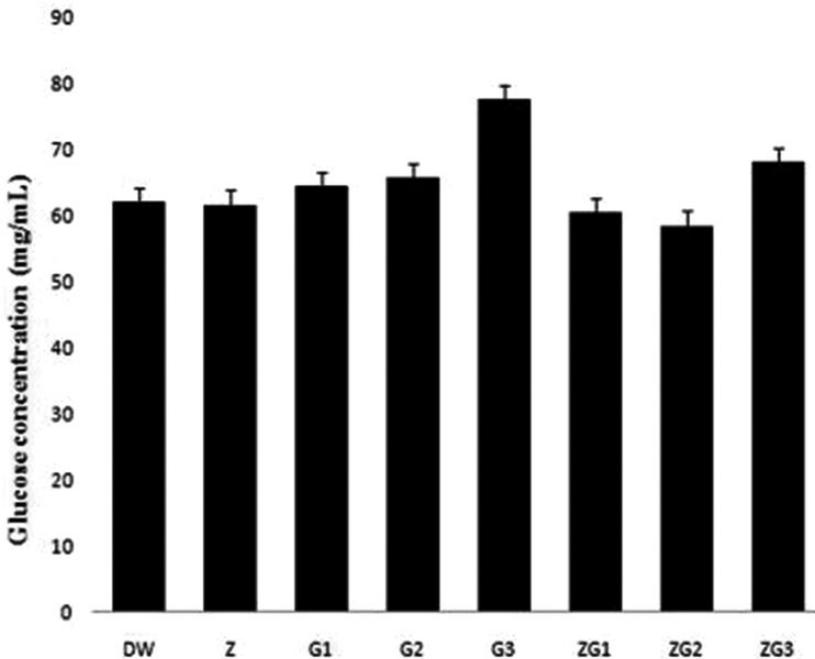
### Effects of treatments on serum insulin levels

There was no significant difference ( $p > 0.05$ ) in serum insulin levels between the treatment groups. An increase in serum insulin levels was observed in the ZG1 and G2 groups, compared with the levels in the DW group (30% and 33%, respectively; Figure 2).

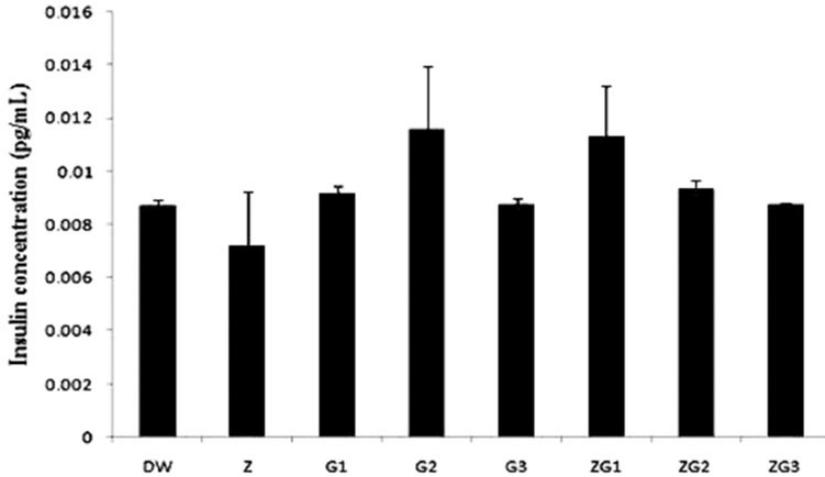
### Histopathological findings

There were no visible lesions in the pancreatic tissues of rats from group I (DW) or group II (Z) (Figure 3a). Tissues from rats

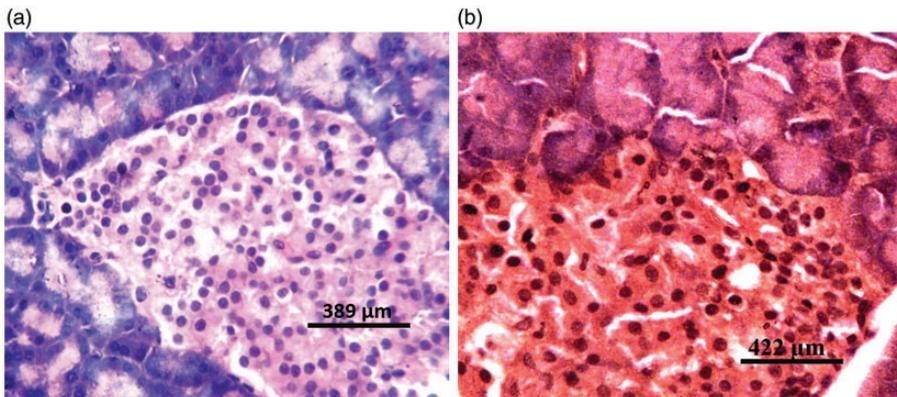
in group III (G1) and group IV (G2) showed degeneration of both pancreatic acinar cells and islets of Langerhans (Figures 4a and 5a, respectively). Severe degeneration of both pancreatic acinar cells and islets of Langerhans was observed in tissues from rats in group V (G3) (Figure 6a). Tissues from groups VI (ZG1), VII (ZG2), and VIII (ZG3) did not exhibit visible lesions (Figures 7a, 8a, and 9a, respectively). Histochemical analysis revealed morphologically normal islets of Langerhans in groups DW, Z, ZG1, ZG2, and ZG3 (Figures 3b, 7b, 8b, and 9b), whereas samples from groups G1, G2, and G3 revealed regions of depopulated and less deeply stained cells in the islets of Langerhans (Figures 4b, 5b, and 6b, respectively).



**Figure 1.** Blood glucose levels in male Wistar rats treated with 2 mL/kg distilled water (DW), 50 mg/kg zinc (Z), 14.4 mg/kg glyphosate-based herbicide (Bushfire<sup>®</sup>) (G1), 375 mg/kg Bushfire<sup>®</sup> (G2), 750 mg/kg Bushfire<sup>®</sup> (G3), 50 mg/kg zinc + 14.4 mg/kg Bushfire<sup>®</sup> (ZG1), 50 mg/kg zinc + 375 mg/kg Bushfire<sup>®</sup> (ZG2), or 100 mg/kg zinc + 750 mg/kg Bushfire<sup>®</sup> (ZG3) for 36 weeks by gavage.



**Figure 2.** Serum insulin levels in male Wistar rats treated with 2 mL/kg distilled water (DW), 50 mg/kg zinc (Z), 14.4 mg/kg glyphosate-based herbicide (Bushfire<sup>®</sup>) (G1), 375 mg/kg Bushfire<sup>®</sup> (G2), 750 mg/kg Bushfire<sup>®</sup> (G3), 50 mg/kg zinc + 14.4 mg/kg Bushfire<sup>®</sup> (ZG1), 50 mg/kg zinc + 375 mg/kg Bushfire<sup>®</sup> (ZG2), or 100 mg/kg zinc + 750 mg/kg Bushfire<sup>®</sup> (ZG3) for 36 weeks by gavage.

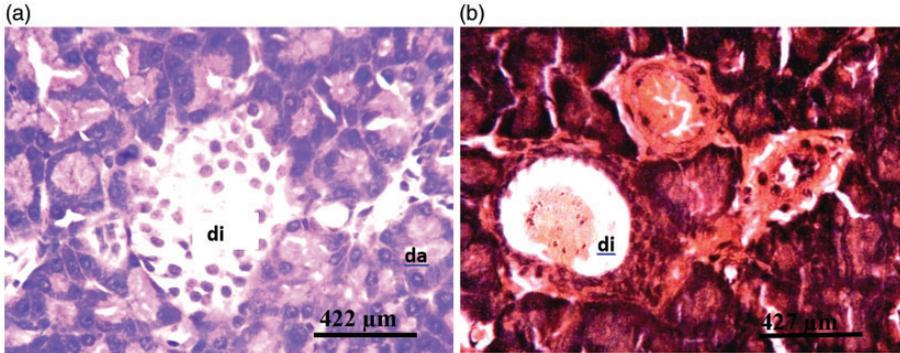


**Figure 3.** Photomicrographs of pancreas of rat administered distilled water (DW) for 36 weeks by gavage, showing no visible lesions. (a) Haematoxylin and eosin staining; and (b) aldehyde fuchsin staining.

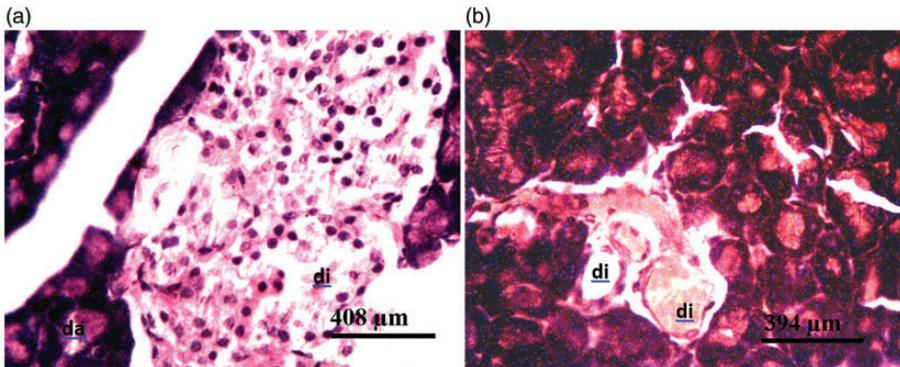
## Discussion

We found a relative increase in blood glucose levels in glyphosate-exposed rats that did not reach statistical significance. This increase may be attributable to the oxidative damage induced in the pancreas by glyphosate. Stress is known to activate the hypothalamic-pituitary-adrenal (HPA) axis

and the sympathetic nervous system, resulting in hyperglycaemia.<sup>25–28</sup> Activation of the HPA axis causes increased secretion of glucocorticoids from the adrenal cortex, eventually resulting in increased gluconeogenesis. The activation of the HPA axis has also been reported to impair glucose uptake in skeletal muscle.<sup>29</sup> Similarly, the stimulation of the sympathetic nervous system



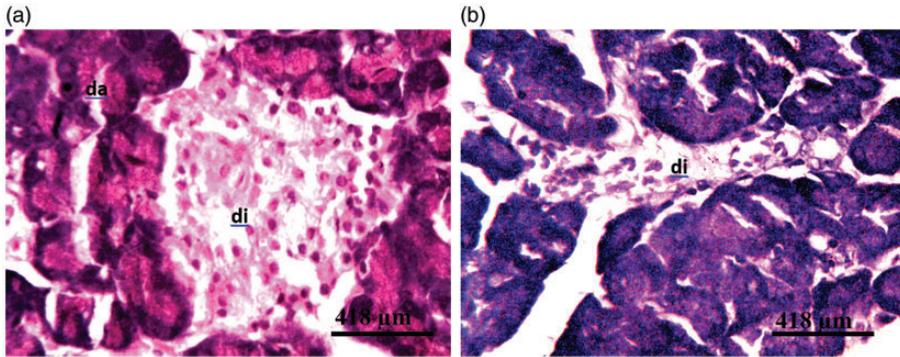
**Figure 4.** Photomicrographs of pancreas of rat administered 14.4 mg/kg of the glyphosate-based herbicide Bushfire® for 36 weeks by gavage, showing (a) moderate degeneration of the islets of Langerhans (di) and degeneration of pancreatic acinar cells (da) (haematoxylin and eosin staining) and (b) severe cystic degeneration of the cells of the islets of Langerhans (di) (aldehyde fuchsin staining).



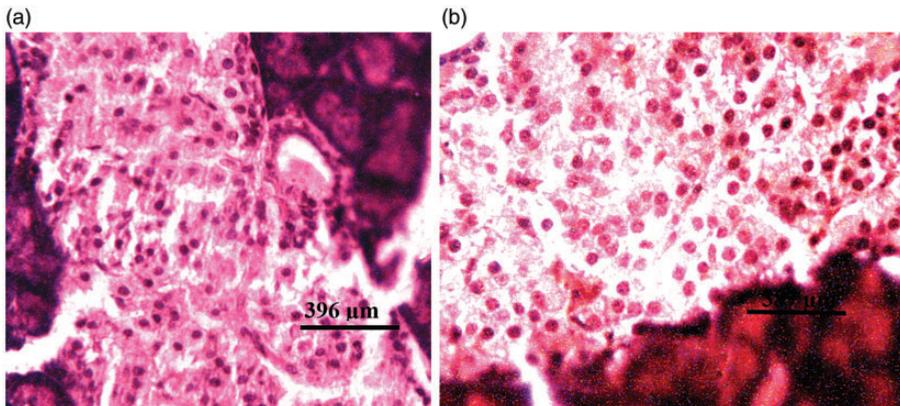
**Figure 5.** Photomicrographs of pancreas of rat administered 375 mg/kg of the glyphosate-based herbicide Bushfire® for 36 weeks by gavage, showing (a) moderate degeneration of the islets of Langerhans (di) and degeneration of pancreatic acinar cells (da) (haematoxylin and eosin staining) and (b) severe degeneration of the cells of the islets of Langerhans (di) (aldehyde fuchsin staining).

under stress conditions has been reported to lead to increased secretion of catecholamines, glucagon, and growth hormone, promoting gluconeogenesis, glycogenolysis, insulin resistance, and hyperglycaemia.<sup>30,31</sup> Previous studies have shown that organophosphate pesticides induce insulin resistance by inhibiting glucose transport in skeletal muscle via alterations in the insulin signalling pathway.<sup>32,33</sup> It can therefore be deduced that the increased glucose levels

observed in this study, along with the corresponding increase in insulin secretion, may be attributable to insulin resistance induced by activation of the HPA axis and/or oxidative stress associated with reduced peripheral tissue uptake of glucose and a chronic exposure. Zinc supplementation exerted a protective effect on serum glucose levels, possibly by preventing oxidative stress and decreasing insulin resistance.<sup>17,34</sup>



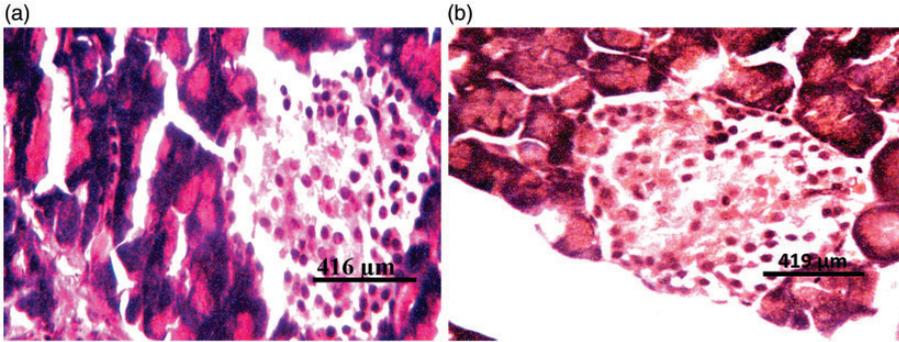
**Figure 6.** Photomicrographs of pancreas of rat administered 750 mg/kg of the glyphosate-based herbicide Bushfire<sup>®</sup> for 36 weeks by gavage, showing (a) severe degeneration of the islets of Langerhans (di) and acinar cells (da) (haematoxylin and eosin staining) and (b) severe degeneration of the islets of Langerhans (di) (aldehyde fuchsin staining).



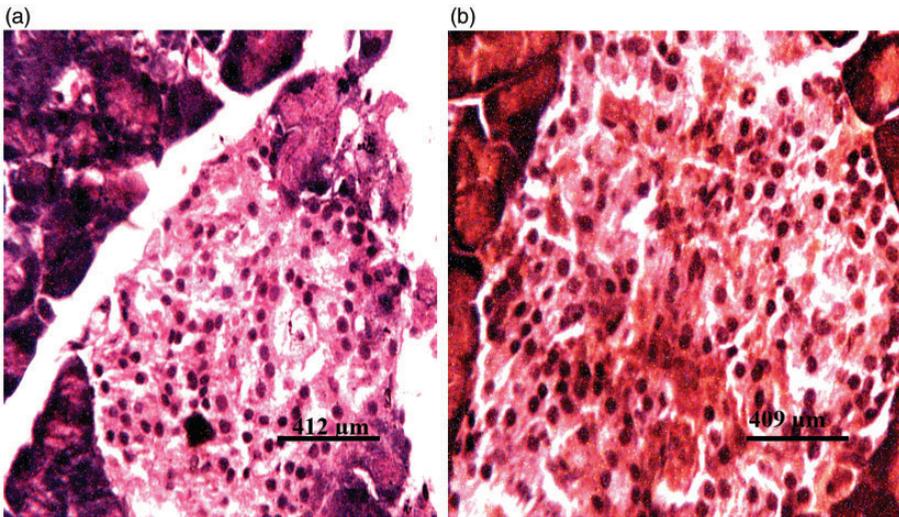
**Figure 7.** Photomicrographs of pancreas of rat administered 50 mg/kg zinc and 14.4 mg/kg of the glyphosate-based herbicide Bushfire<sup>®</sup> for 36 weeks by gavage, showing no visible lesions. (a) Haematoxylin and eosin staining; and (b) aldehyde fuchsin staining.

Rats exposed to 14.4 and 375 mg/kg glyphosate exhibited a relative increase in serum insulin levels, perhaps because of oxidative damage, while rats exposed to 750 mg/kg glyphosate did not. This finding may be due partly to the degenerative changes in the islets of Langerhans that were observed in these groups; the damage would be expected to limit insulin secretion. Previous studies have shown that organophosphate pesticides can elevate insulin

levels and lead to insulin resistance by inhibiting glucose transport and dysregulating the insulin signalling pathway.<sup>32,33</sup> Zinc treatment alone caused a relative decrease in serum insulin levels, possibly because of the pro-oxidant effect of zinc. Zinc supplementation prior to treatment with the lowest glyphosate dose resulted in an apparent increase in serum insulin levels compared with levels in the control group. The pro-oxidant effect of zinc has been



**Figure 8.** Photomicrographs of pancreas of rat administered 50 mg/kg zinc and 375 mg/kg of the glyphosate-based herbicide Bushfire® for 36 weeks by gavage, showing no visible lesions. (a) Haematoxylin and eosin staining; and (b) aldehyde fuchsin staining.



**Figure 9.** Photomicrographs of pancreas of rat administered 100 mg/kg zinc and 750 mg/kg of the glyphosate-based herbicide Bushfire® for 36 weeks by gavage, showing no visible lesions. (a) Haematoxylin and eosin staining; and (b) aldehyde fuchsin staining.

documented in earlier studies,<sup>35,36</sup> but zinc supplementation in the groups that received 375 and 750 mg/kg glyphosate restored serum insulin levels to near normal.

Degeneration of both pancreatic acinar cells and islets of Langerhans were observed, probably as a result of oxidative damage. Similarly, in our previous study,<sup>14</sup> we observed degeneration of pancreatic acinar cells following subchronic (8-week)

exposure to the glyphosate-based herbicide Bushfire® in rats. The damage to the islets of Langerhans observed in this study may be attributable to the increased duration of exposure. Zinc supplementation in the present study prevented any visible histopathological damage, indicating that zinc may exert an ameliorative effect on the pancreas. Zinc has been reported to play an important role in the maintenance of structure,

function, and integrity of biological membranes,<sup>37,38</sup> and to protect sulphhydryl groups against oxidation, thereby stabilising the cellular thiol pools.<sup>39</sup>

We did not conduct an oral glucose tolerance test, which would have determined the rate at which glucose was cleared from the blood, so we could not verify whether the rats had developed insulin resistance. In addition, we did not identify whether  $\beta$ -cells or  $\alpha$ -cells in the islets of Langerhans were most affected by the exposure. This information would have been beneficial in elucidating why serum insulin levels increased as blood glucose increased.

In summary, chronic exposure to the glyphosate-based herbicide Bushfire<sup>®</sup> can alter blood glucose homeostasis and influence insulin secretion in rats by damaging pancreatic islet and acinar cells, and zinc supplementation can ameliorate these effects.

#### Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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